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RESEARCH ARTICLE

Reproductive biology of the mutton hamlet on the coast of Pernambuco, northeastern Brazil

Biologia reprodutiva do sapé pintado no litoral de Pernambuco, Brasil

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Abstract

This paper focuses on the reproductive biology of the Mutton Hamlet, *Alphestes afer*, a fish belonging to the family Epinephelidae, and aims to provide information on the main reproductive parameters of the species on the north coast of the state of Pernambuco, northeastern Brazil. From a total of 783 individuals, collected between March 2007 and June 2010, and from February to November 2014, the gonads of 322 fishes (235 females and 87 males) were analyzed, for the identification of sex and maturational stages. Females were more abundant and larger than males, with a sex ratio of 2.4 females: 1 male, during the reproduction season. The size at first maturity (L_{50}) was estimated at 16.8 cm total length for females, and 16.2 cm for males. The gonadosomatic index (GSI) indicated that a reproductive cycle occurs all year round, while reproduction takes place from June to November. The fecundity of *Alphestes afer* ranged from 105,317 to 270,192 oocytes, averaging 173,458 oocytes. The females are more abundant than males due to the process of protogyny and also because individuals are regularly captured at growth stages above the $L_{50,}$ being a positive aspect for the sustainability of the fishery. According to the GSI, the species presents a split-type spawning with two peaks occurring throughout the year.

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Resumo

Este trabalho aborda a biologia reprodutiva do sapé pintado, *Alphestes afer*, um peixe teleósteo pertencente à família Epinephelidae e objetiva aportar informações sobre os principais parâmetros reprodutivos da espécie no litoral norte de Pernambuco, Brasil. Um total de 783 indivíduos foram coletados, entre os meses de março de 2007 a junho de 2010 e de fevereiro a novembro de 2014, tendo sido analisadas 322 gônadas e identificadas quanto ao sexo e estágios maturacionais (235 fêmeas e 87 machos). As fêmeas foram mais abundantes e maiores que os machos, com uma proporção sexual de 2,4 fêmeas: 1 macho no período reprodutivo. O tamanho de primeira maturação foi estimado em 16,8 cm de comprimento total para as fêmeas e 16,2 cm para os machos. O Índice Gonadossomático (IGS) indicou que o ciclo reprodutivo ocorre durante todo o ano, e a reprodução, de junho a novembro. A fecundidade do *Alphestes afer* variou de 105.317 a 270.192 ovócitos, com média de 173.458 ovócitos. As fêmeas são mais frequentes que os machos devido ao processo da protoginia, além do fato de os indivíduos serem capturados acima do L_{50}, sendo esse um aspecto positivo para a sustentabilidade da pesca. De acordo com o IGS, a espécie apresenta uma desova do tipo parcelada com dois picos ocorrendo ao longo do ano.

Keywords

Epinephelidae, fecundity, reproduction, spawning

Palavras-chave

Desova, Epinephelidae, fecundidade, reprodução

Introduction

The family Epinephelidae includes marine rocky and coralline fishes from tropical and subtropical areas, which are commercially important for artisanal fisheries, composing an important portion of their profit (Heemstra and Randall 1993).

Alphestes afer is frequently found in reefs, near coasts or islands. The species is characterized by its robust, small, and laterally compressed body, brown colored with orange little dots, and dark-brown spots, with small and hard scales, rarely reaching a total length of 30 cm (Craig et al. 2006). *Alphestes afer* is a diachronic hermaphrodite species, changing sex in a protogynic mode, in two different ways: either immature females transform into primary males, or females at mature, spent, or resting stages transform into secondary males (Marques and Ferreira 2011).

Alphestes afer populations occur in the Atlantic Ocean, in south-west Florida (USA), Bermuda, the Gulf of Mexico, the Caribbean Sea, Bahamas, Cuba, and other Antilles, Panama, and Western Africa. This species has also been rediscovered in São Tomé and Principe, and in the Gulf of Guinea, in the Eastern Atlantic. In Brazil, it is distributed along the entire coast, from Amapá to Santa Catarina (Craig et al. 2006; Hostim-Silva et al. 2006; Wirtz et al. 2007; Sampaio and Nottingham 2008). In Northeast Brazil, species such as the mutton hamlet, *Alphestes afer*, the coney, *Cephalopholis fulva*, and the catfish, *Epinephelus adscencionis*, are the most common groupers caught by the artisanal fisheries.

The diversity of organisms associated with reef ecosystems is threatened by habitat degradation caused by pollution, silting, industrialization, agriculture, and

mainly, overfishing (Floeter et al. 2006). Investigations about reef fishes, such as those of the family Epinephelidae, which occupy a high level in the trophic chain, are important not just because of their high susceptibility to overfishing, but also due to the crucial ecological role they play in reef ecosystems (Sampaio and Nottingham 2008).

In this study, we analyzed the main reproductive parameters of the mutton hamlet, in order to achieve a better understanding of its population dynamics, useful for the development of management strategies for a sustainable exploration of the stocks.

Material and methods

This study was carried out in two phases, from March 2007 to November 2010, and from February to November 2014. During these periods, sample fishes were collected monthly from fishermen at Itamaracá, north of the state of Pernambuco, in northeastern Brazil. The individuals were caught by artisanal fishing boats using fish traps, which were submersed to depths ranging from 30 to 50 m. All samples were stored on ice and taken to the laboratory, where total weight (TW), gutted weight (GT), and total length (TL) were measured. Gonads were removed and submitted to histological processing and analysis. A small section of the gonads of all the sampled specimens was dehydrated using alcohol, cleared with xylene, embedded in paraffin, sectioned on the microtome (5 μm thick), and then stained with hematoxylin and eosin (Mackie and Lewis 2001).

From a total of 783 individuals collected, 322 had the gonads analyzed (235 were females, and 87, males) for the identification of sex and maturational stages, using both macro- and microscopic developmental characteristics (adapted from Brown-Peterson et al. 2011). The reproductive development of a fish comprises five reproductive stages, both for females and males: immature, developing, mature, spent, and resting. The gonadosomatic index (GSI) was calculated as

$$GSI = \frac{GW}{(TW-GW)} \text{x} 100,$$

where: GW = gonad weight (g) and TW = total weight (g). In this procedure, immature individuals were not included.

The size at first sexual maturity (L_{50}) was estimated using a logistic curve (Brown and Rothery 1993), comparing the relative maturation of individuals and their total length; immature specimens were excluded. The period of spawning was evaluated by the monthly distribution of frequencies of the different maturational stages, using the GSI of mature females as a reference (Vazzoler 1996). To evaluate the sexual ratio (both monthly and by length class), 667 specimens were used (503 females and 164 males). The statistical significance of differences in sexual proportion between males and females was determined through a Chi-squared test (X^2; $p<0.05$).

Fecundity was estimated by the gravimetric method proposed by Hunter et al. (1985), according to which a sample of 0.5 g is removed from a median portion of

six mature ovaries to count the actual number of hydrated oocytes. Then, results are applied to the equation:

$$Fb = \frac{\text{n.Wg}}{w},$$

where: Fb is the total number of hydrated oocytes in a gonad, n is the total number of hydrated oocytes in a sample, Wg is the weight of both ovaries and w is the weight of the sample.

Results

In this study, a total of 783 individuals was sampled, and 667 of them had their sex identified: 503 were females, and 164 were males. A chi-squared test (X^2, $p<0.05$, degrees of freedom = 1) showed a significant difference in the sex ratio for the entire period of time, with a predominance of females (2.4:1, female to male ratio) in the reproduction season. Females were significantly more abundant in all months, except for August ($p = 0.07$) and November ($p = 0.27$; Table 1).

In relation to size, the TL of females varied from 15.0 to 27.0 cm, and of males from 15.1 to 23.8 cm, with a mode for both sexes in the length class of 20–22 cm (Figure 1).

Among the 235 females examined for gonad maturation, 19 were immature, 66 were developing, 78 were mature, 15 were spent, and 57 were resting. In the developing stage, the oocytes were already developing, but were still not ready for spawning. Alveolar-cortical oocytes (AC) and oocytes in primary vitellogenesis (Vtg1) were present in this stage. In the ovaries in the mature stage, the presence of germinal vesicle breakdown (GVBD) and post-ovulatory follicles (POF) was observed, while those in spent stage presented atresic oocytes (A). Females in resting stage, showed thin-walled ovaries not reproductively active, that presented blood strings (BS) and primary growth (PG; Figure 2).

Table 1. Monthly sex ratios for *Alphestes afer* populations sampled from March 2007 to June 2010 and from February to November 2014, in the northern coast of Pernambuco, northeastern Brazil. *not statistically different.

Month	Males	Females	TOTAL	X^2	P
Jan	11	46	57	21.49	3.55×10^{-6}
Feb	26	56	82	10.97	9.25×10^{-4}
Mar	5	25	30	13.33	2.61×10^{-4}
Apr	21	80	101	34.46	4.35×10^{-9}
May	11	70	81	42.97	5.56×10^{-11}
Jun	17	49	66	15.51	8.21×10^{-5}
Jul	8	25	33	8.75	3.10×10^{-3}
Aug	8	17	25	3.24*	0.07
Sep	19	45	64	10.56	1.16×10^{-3}
Oct	16	45	61	13.78	2.06×10^{-4}
Nov	12	18	30	1.2*	0.27
Dec	10	27	37	7.81	5.20×10^{-3}
TOTAL	**164**	**503**	**667**	**172.29**	$\mathbf{<2.20 \times 10^{-16}}$

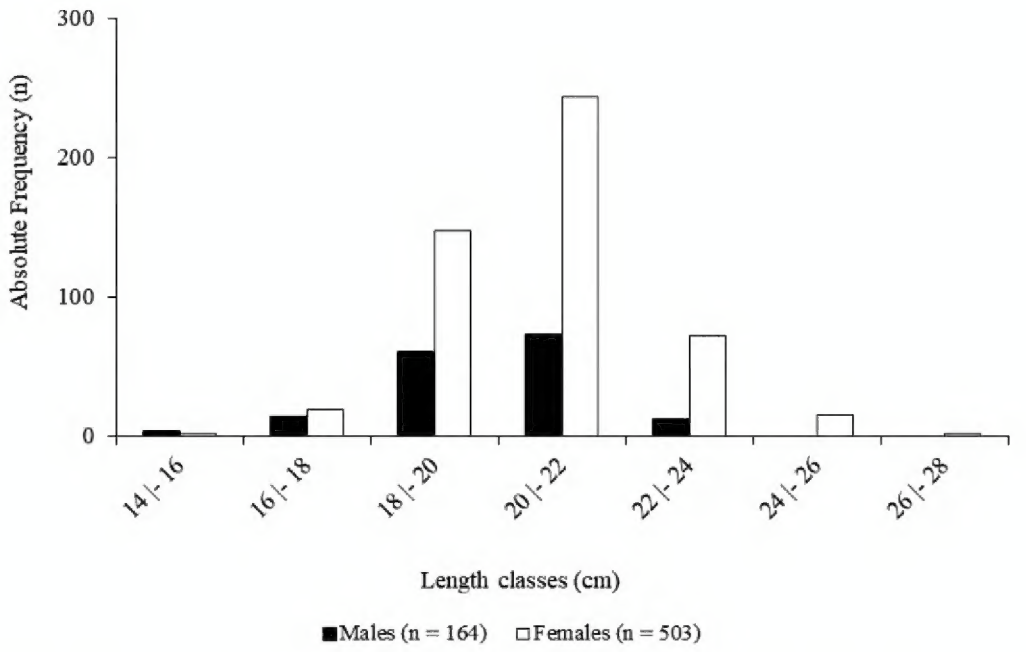

Figure 1. Absolute frequency distribution of length classes for males and females of mutton hamlet, caught to the north of Pernambuco, northeastern Brazil, from March 2007 to June of 2010 and from February to November 2014.

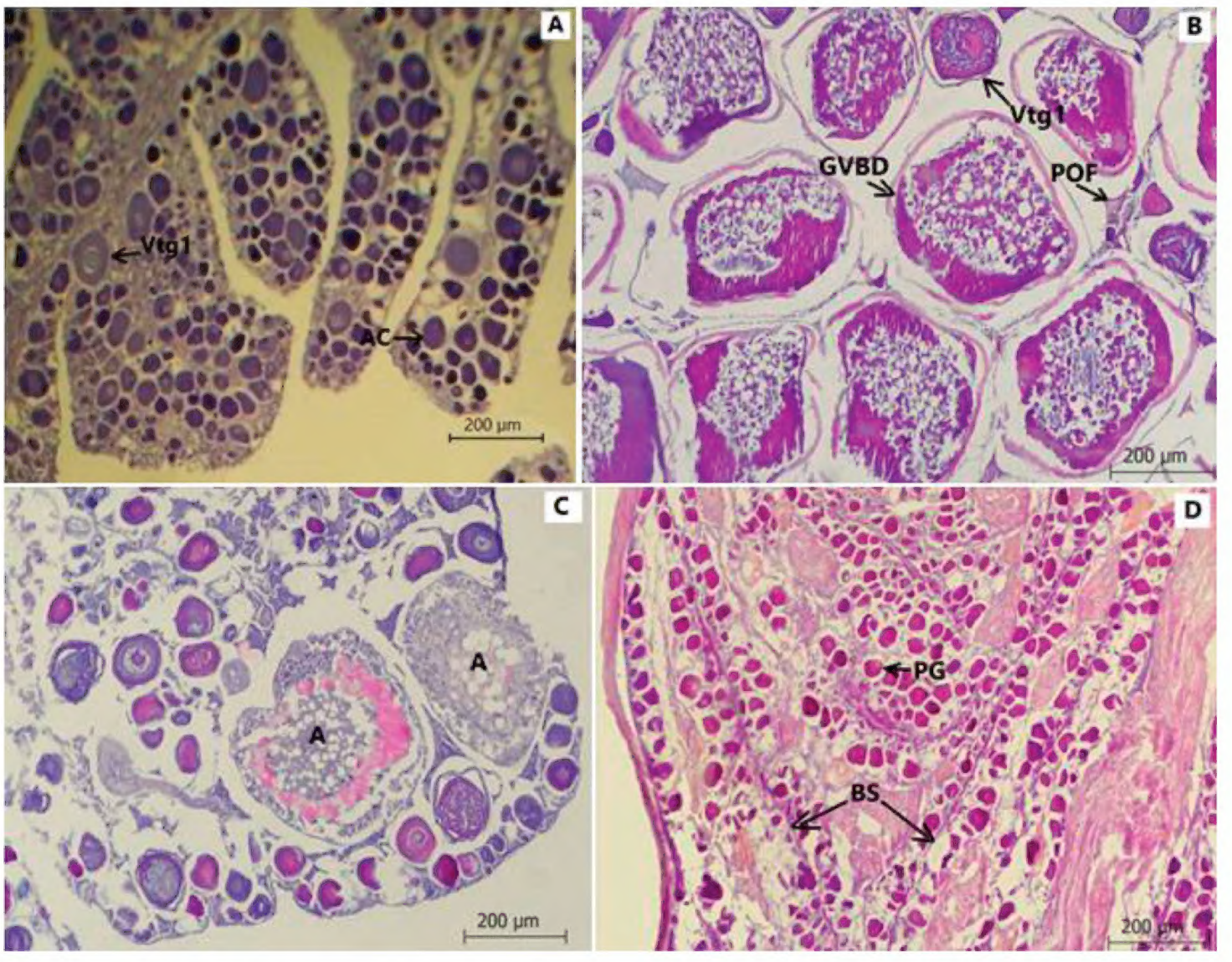

Figure 2. Ovaries of *Alphestes afer* in different maturational stages: Developing (A); Mature (B); Spent (C); Resting (D). A = atresic oocytes; AC = alveolar cortical; BS = blood strings; GVBD = germinal vesicle breakdown; PG = Primary growth POF = post-ovulatory follicle; Vtg1 = primary vitellogenesis and Vtg3 = tertiary vitellogenesis.

The developing stage occurred in almost all months of the year, except for April and November. Females with resting ovaries did not occur in August and December, while spent females occurred in January, February, April, September, and November. Finally, mature females were found in April and from July to January (Figure 3A). Among the 87 males examined for maturation, 5 were immature, 34 were developing, 27 were mature, with testicles reaching their biggest size, and occupying up to 90% of the abdominal cavity, 6 were spent, and 15 were resting. The developing

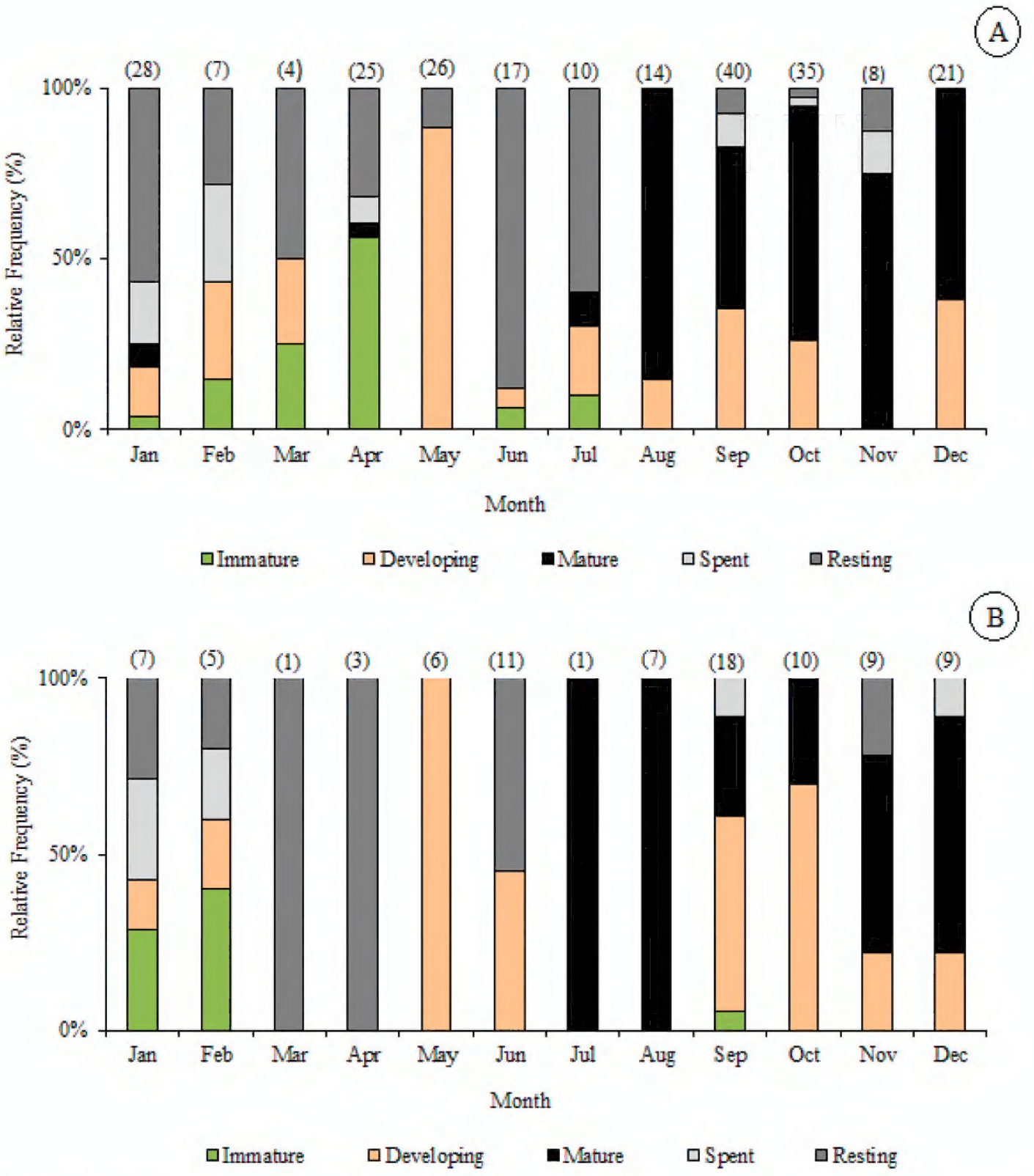
A
(28) (7) (4) (25) (26) (17) (10) (14) (40) (35) (8) (21)
100%
50%
0%
Relative Frequency (%)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Immature Developing Mature Spent Resting
B
(7) (5) (1) (3) (6) (11) (1) (7) (18) (10) (9) (9)
100%
50%
0%
Relative Frequency (%)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Immature Developing Mature Spent Resting

Figure 3. Monthly distribution of maturational stages of females (A) and males (B) of *Alphestes afer* caught to the north of Pernambuco, northeastern Brazil, from March 2007 to June 2010 and from February to November 2014.

stage was the most commonly found (20.7%). Immature males were observed only in January, February, and September, while mature males were found from July to December (Figure 3B).

The highest monthly average values of GSI for females were observed in August and November (168 and 118, respectively), while the lowest values were found in February. For males, the highest values were noticed in July and August (106 and 112, respectively), while the lowest occurred in March (Figure 4). According to the GSI, there are two reproductive peaks for females, and a sharp peak with a slow decline for males, each year. These data indicate a possible split-type spawning for *Alphestes afer* populations in the littoral zone north of Pernambuco.

The average L_{50} was estimated at 16.8 cm TL for females (Figure 5A) and at 16.2 cm TL for males (Figure 5B). Out of the 235 females and 87 males analyzed for maturation, 92% females (216), and 94% males (84) were adults. The fecundity of six mature females of *A. afer* with a TL of 19.9 to 21.2 cm varied from 105,317 to 270,192 mature oocytes with an average of 173,458. Absolute fecundity increased linearly with gonad weight (Figure 6). The relationship between fecundity and female's length showed variations. It can be observed that the smallest female, 19.9 cm long, presented a higher fecundity, with 175,959 oocytes, than the largest female, 21.2 cm long, which had 164,000 oocytes. The highest value of fecundity was recorded in a 20.5 cm long female with 270,192 mature oocytes (Figure 7).

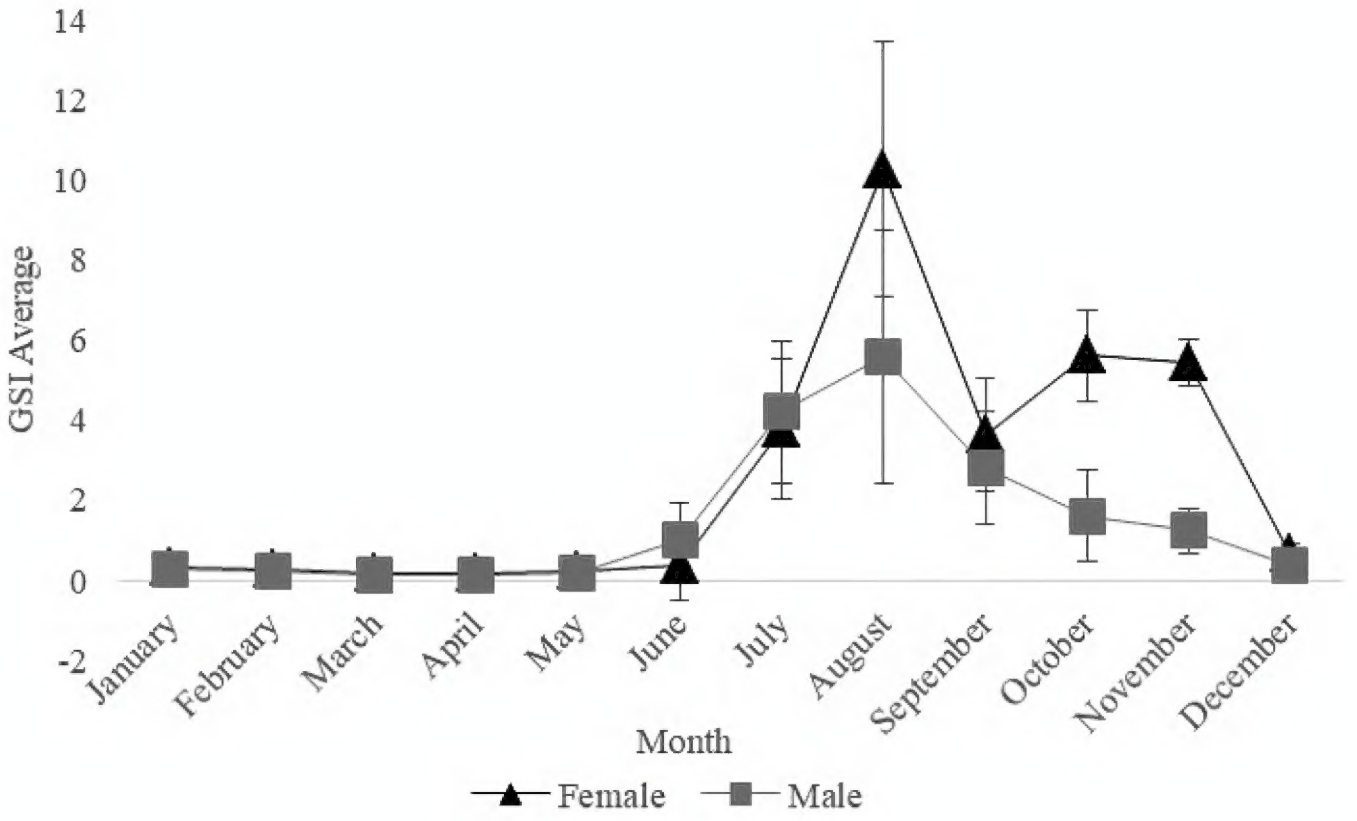

Figure 4. Monthly average for gonadosomatic index (GSI) of males and females of *Alphestes afer*, caught to the north of Pernambuco, northeastern Brazil, from March 2007 to June 2010 and from February to November 2014.

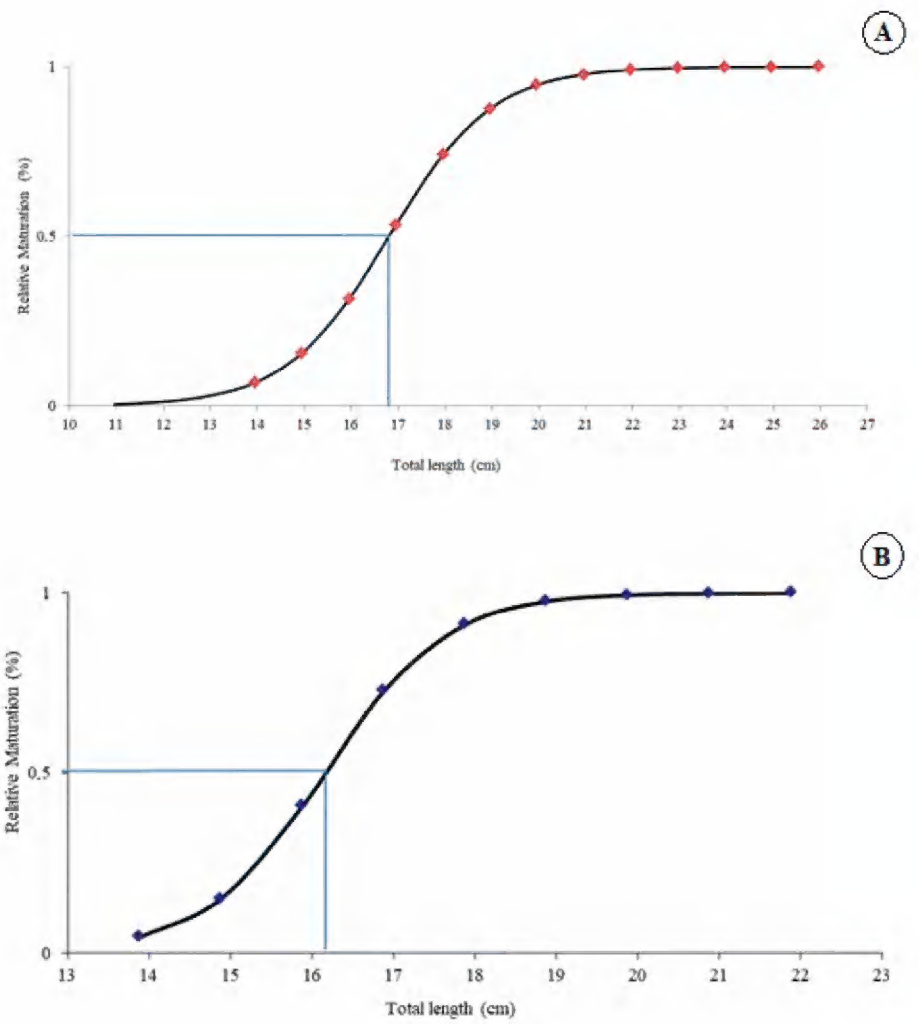

Figure 5. Size at first sexual maturity for females (A) and males (B) of *Alphestes afer*, caught to the north of Pernambuco, northeastern Brazil, from March 2007 to June 2010 and from February to November 2014.

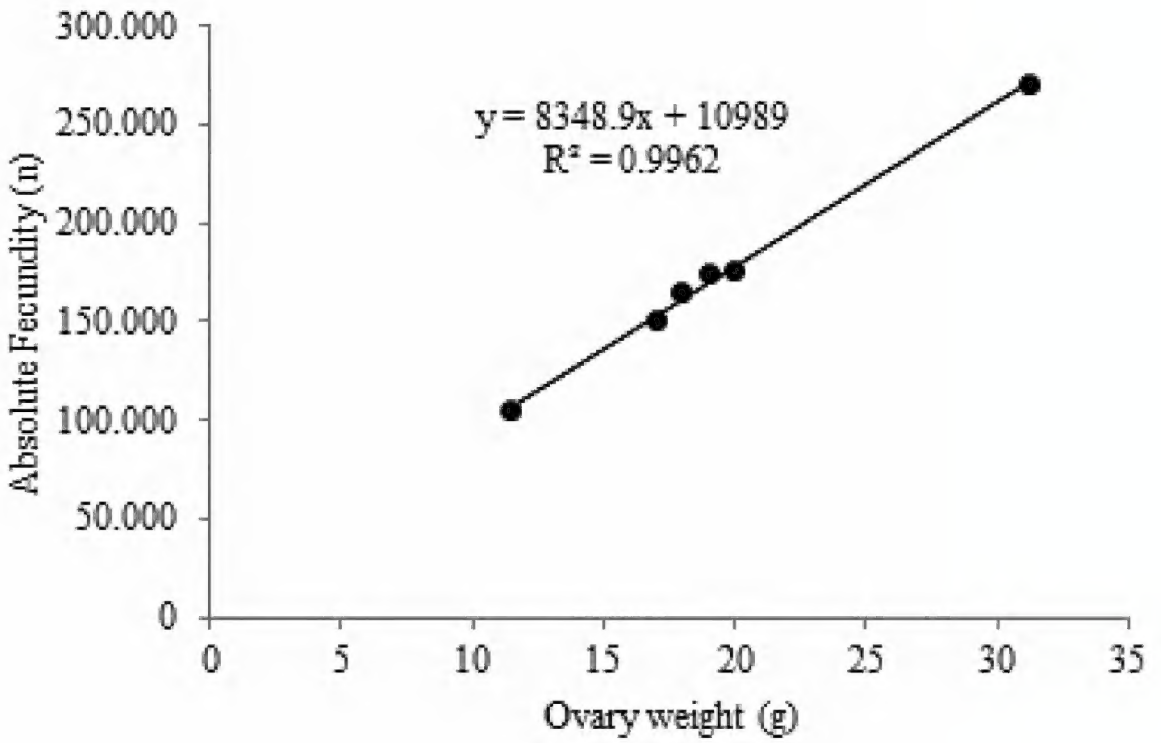

Figure 6. Relationship between absolute fecundity and ovary weight of *Alphestes afer* caught to the north of Pernambuco, northeastern Brazil, from March 2007 to June 2010 and from February to November 2014.

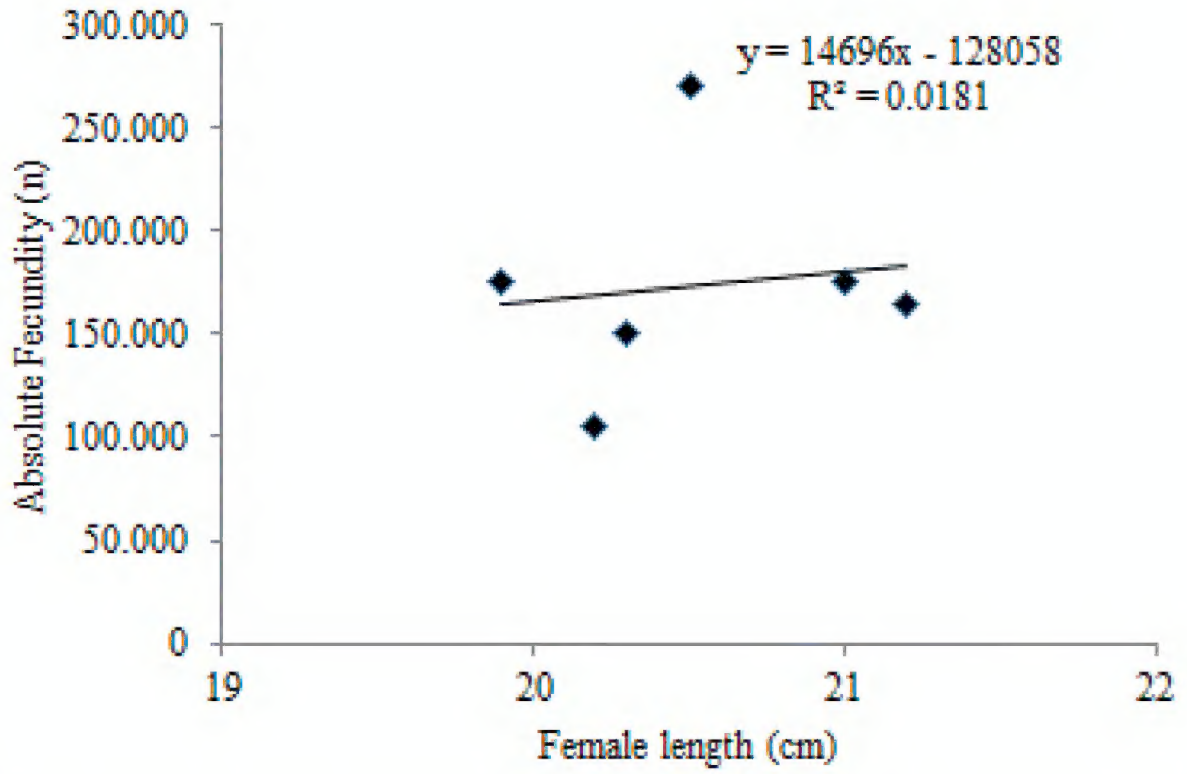

Figure 7. Relationship between absolute fecundity and female length of *Alphestes afer* caught north of Pernambuco, northeastern Brazil, from March 2007 to June 2010 and from February to November 2014.

Discussion

Females of *A. afer* from the northern coast of Pernambuco were about two times more abundant than males (2.4:1, female to male ratio) in the reproduction season, with the highest abundance occurring in the largest length classes. This is likely a consequence of the protogyny exhibited by the species, *i.e.* most specimens are born as females and after the second or third reproduction (adult individuals from the largest length classes), they change their sex (Heemstra and Randall 1993). Such a sex change was also mentioned by Marques and Ferreira (2011) in a reproductive study of *A. afer* done in the south coast of Pernambuco. They observed immature bisexual and transitional individuals (presenting both ovarian and spermatic tissues), with immature females becoming immature males.

The length-frequency distribution found in this study could probably not express the real size structure of the population, due to fishing gear (fish traps) selectivity. Moreover, it is probable that this species presents a different length-frequency distribution by depth, with smaller and larger individuals not occurring in depths where the traps were set (approximately 40 m). However, even with fishing gear selectivity, the total length ranges registered in this study for *A. afer*, are within the generally described ranges for this genus, with sizes varying from 13 to 33 cm for species like *A. afer*, *A. immaculatus*, and *A. multigutattus* (Heemstra and Randall 1993).

The highest monthly average values of GSI for females compared to males, during the spawning months, indicates that females present heavier gonads, mainly because they have larger body lengths than males. The monthly mean variation of

the GSI for both males and females, with its highest values in August, indicates a seasonal reproductive cycle with spawning occurring mainly during the second semester. That is an intermediary period from the end of the rainy season (finishing in July) and the start of the dry season (finishing in December). According to the Agência Pernambucana de Águas e Clima (APAC), the rainy season in the littoral of Pernambuco occurs from March to July, but during August, significant rainfall can still occur in this region (APAC 2014). Therefore, it is likely that *A. afer* starts to spawn at the end of the rainy season of Pernambuco to take advantage of the high concentration of food. The effect of river discharges (nutrients) on the coastal environment generates high concentrations of primary biomass soon after the rainy season, between September and October, initiating a spring bloom in the Pernambuco region (Ressurreição et al. 1996). According to Araújo (2009), this correlation with the period of greatest water supply is a reproductive tactic to potentiate the success of spawning, since there is greater food availability after rainfall, thus reducing the threat of predation of eggs and larvae. This result was similar to the ones found by Ferreira (1993) for two species of the family Epinephelidae, *Plectropomus maculatus* and *P. leopardos*, in Australian reefs, where they present a reproductive period from September to November. Gaspare and Bryceson (2013), analyzing *Epinephelus malabaricus* from Mafia Island, observed mature females from September to December and mature males from September to February. According to these authors, the spawning occurs in this period of the year, right after the rainy season, due to an increase in the concentration of nutrients in coastal areas, promoting a greater development of zooplankton, which serve as food for the larvae of *E. malabaricus*.

The L_{50} for species of the family Epinephelidae are commonly reached when individuals attain about 50% of their maximum total length (Sadovy 1996). The L_{50} value estimated here for females of *A. afer* (16.8 cm) seems to conform to that, being also close to the one found by Marques and Ferreira (2011) in the same region (18 cm). Therefore, the length frequency distribution found for the species, indicates that the majority of individuals caught were bigger than the size at first sexual maturity, with very few immature specimens being present in the catches, which is a positive aspect for the sustainability of the fishery.

The presence of post-ovulatory follicles in the mature stage is indicative of continuous oocyte recruitment during the spawning season. In this study, the average fecundity was close to the value observed by Thompson and Munro (1978) for four species of Epinephelidae (mean fecundity of 160.000 oocytes per female). According to Vazzoler (1996), female fecundity is an important parameter that depends more on female size than on age, increasing with growth. However, this affirmation was not observed in the six females of *A. afer* analyzed, where the highest value of fecundity was obtained from the smallest female, indicating that higher values of fecundity, has more to do with the age of the female than its growth. According to Hixon et al. (2014), it is necessary to keep older individuals in the population, the 'big breeders' (big, old, fat, fecund, female fish or BOFFFFs), since they are individuals with high fertility rates, crucial for the conservation of the species, contributing to the maintenance of the population's biomass.

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